Arabian Journal of Chemistry (2011) 4, 147–152

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Development of a selective fluorimetric technique for rapid trace determination of zinc using 3-hydroxyflavone

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Received 18 February 2010; accepted 20 June 2010
Available online 25 June 2010

KEYWORDS

Zinc;
Spectrofluorimetry;
Hydroxyflavone;
Trace levels

Abstract A sensitive and a selective spectrofluorimetric method have been developed for the rapid determination of trace levels of zinc. The method is based on complex formation between zinc and 3-hydroxyflavone (3HF), which displays an intense emission signal around 478 nm. The analytical performance of the method was examined by considering the factors that affect the complex formation such as pH, mole ratio of the metal and solvent type. The optimum conditions for the complex formation were metal to ligand stoichiometric ratio of 1:1 at pH 7.5 with 0.1 M Tris buffer. Under these conditions the detection limit attained was 1.5 ppb. The method was appropriately validated and yielded relative standard deviations of less than 2% ($n = 5$), which was considered acceptable. It was successfully applied to the trace determination of zinc in drinking water, hair shampoo and pharmaceutical samples.

1. Introduction

Zinc is a mineral that is essential for a healthy immune system and has a great importance for humans, plants and animals. It plays a significant role in cell replication, protein synthesis, gene expression and cell division.

The deficiency of zinc in humans causes several disorders such as growth retardation, diarrhea, malfunctioning of wound healing and dermatitis (Scherz and Kirchhoff, 2006). On the other hand, zinc can be toxic when exposures exceed physiological needs. Elevated levels of zinc are known to play a major role in cell damage following stroke (Yasui, 1987) and may be a risk factor in Alzheimer's disease (Atwood et al., 1999; Danscher et al., 1997).

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Peer review under responsibility of King Saud University.
doi:10.1016/j.arabjc.2010.06.029

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The concentration of zinc in natural water is usually very low and can span a wide range from 10^{-10} M to 10^{-6} M (DeZuane, 1990). Concentrations of zinc greater than 5.0 mg L^{-1} affect the potable nature of water. Zinc occurs in hair at relatively high levels of about 100–300 ppm and its determination can be useful for clinical and forensic purposes (Clanet et al., 1982).

Zinc compounds are widely used in the pharmaceutical industry in the manufactures of dermatological and antiseptic disinfectant agent. They form an important ingredient in the preparation of mouthwash, multivitamins preparation, insulin and ophthalmic solution. Zinc has become a popular cure for the common cold.

Because of the importance of zinc, simple and sensitive analytical methods for the determination of trace levels of zinc are required. The commonly used analytical methods for the quantitative determination of zinc are graphite furnace atomic absorption spectrometry (GF-AAS) (Burguera-Pascu et al., 2006), flame-AAS (FAAS) (Han et al., 2007), inductively coupled plasma mass spectrometry (ICPMS) (Mestek et al., 2001) and (Nakatsuka et al., 2007), inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Kara et al., 2005) and (Kilian and Pyrzynska, 2003), spectrophotometry (Reddy et al., 2007; Thanasarakhan et al., 2007; Vieira et al., 2008; Säbel et al., 2009) and electroanalytical techniques (Kruusma et al., 2004).

Molecular fluorescence spectroscopy has been widely used for chemical analyses in a number of fields. This is because of its inherent sensitivity, simplicity; ease of automation, cost effectiveness and to some extent selectivity especially for toxic, mutagenic, or carcinogenic substances (Sharma and Schulman, 1999). Zinc like many other metals does not possess intrinsic fluorescence but it can form complexes with many fluorigenic ligands.

There are many examples of ligands which have been used for complex formation with zinc (Ngwendson et al., 2006; Jiang and Guo, 2004; Ressalan and Iyer, 2005; Yu et al., 2007; Al-Kindy et al., 2008.

Recently, our research group has been interested in the development of fluorigenic methods for the sensitive and selective monitoring of metal ions in water (Al-Kindy et al., 2002, 2003, 2006, 2007, 2008). We have observed with interest the fluorescent attribute of 3-hydroxyflavone (3HF), which represents the basic structural moiety of an important group of naturally occurring, biologically active compounds known as flavonoids. The flavonoid compounds occupy a prominent position among the plant polyphenols. Flavones consist of two benzene rings joined together by a three-carbon link which is formed into a γ-pyrone ring. A great number of flavonoids are able to chelate metal ions and often the presence of a 3-hydroxyl group or 5-hydroxyl group permits complex formation involving the carbonyl functional group (Dangleterre et al., 2008).

We report here a novel fluorimetric method for the determination of zinc based on the complex formation between 3HF and zinc ions. Factors affecting complexation such as pH, molar concentration of ligands to metal and solvent type were studied. To the best of our knowledge the analytical application of 3HF for the determination of zinc has not been reported. In addition, matrix effects linked to intrinsic interferences from diverse metal ions associated with the determination of zinc were investigated. Optimal conditions for the successful determination of zinc were ultimately attained and applied to the assay of multivitamin tablets/capsules, tap water and hair shampoo. Validation of the method was achieved using absorption spectroscopy.

2. Experimental

2.1. Apparatus

A Perkin–Elmer (LS55B) luminescence spectrometer equipped with a xenon lamp was used to record both the emission and the excitation spectra using 1 cm quartz cell. The spectra were recorded without any correction for instrumental characteristics. Measurement of pH was made with a JENWAY 3015 pH meter (UK) using a combined electrode system. The pH meter was calibrated daily before use with buffers of pH 4.0, 7.0 and 9.0. Spectra Alt 220FS Atomic Absorption spectrometer from Varian was used to validate the method for determination of zinc in tablets, capsules, hair shampoo and tap water samples.

2.2. Reagents

All reagents and solvents were commercially available and used without further purification. Zinc acetate was purchased from BDH chemicals Ltd. Poole England. 3-Hydroxyflavone 99%, methanol (HPLC grade), 2-amino-2-(hydroxymethyl)-propane-1,3-diol (Tris) were obtained from Sigma–Aldrich. Hydrochloric acid and sodium hydroxide were obtained from Riedel-de Haën. All chemicals used were of analytical reagent grade and were dissolved in high purity water from a Milli-Q system.

2.3. Sample preparation

Seven to ten multivitamin tablets were weighed, pulverized and homogenized using a mortar and a pestle. In the case of capsules their contents were emptied and homogenized. A portion of powder known to contain an equivalent amount of one tablet or capsule was accurately weighed, dissolved in 2 M HCl, heated to near boiling for ten minutes and then diluted to 50.0 mL with Milli-Q water. For the determination of zinc, aliquots of the diluted sample were added to 10 mL volumetric flasks containing appropriate amounts of 3-hydroxyflavone and filled to the mark with 0.1 M Tris buffer of pH 7.5.

A portion of 0.500 g of a shampoo purchased from a local outlet was dissolved in 2 M HCl and heated to near boiling. The mixture was then transferred to a 50 mL volumetric flask and diluted to the mark with deionized water. For the determination of zinc in the sample, individual aliquots were made up in 10-mL volumetric flasks containing 3-hydroxyflavone and filled up to the mark with Tris buffer.

Tap water samples from SQU university campus were spiked with 10 ppm zinc standard. For the determination of zinc in the sample appropriate aliquots of the diluted solutions were mixed with the ligand and the fluorescence intensity was measured at pH 7.5 Tris buffer.

3. Results and discussion

3.1. Spectral characteristics of Zn-3HF complex in methanol

The fluorescence spectra of 3HF (1.0×10^{-7} M) in methanol and its zinc complex is shown in Fig. 1. 3HF exhibited a fluorescence signal at λ_{em} 453 nm when excited at 378 nm. It is clear from Fig. 1 that when Zn^{2+} ions were added to the

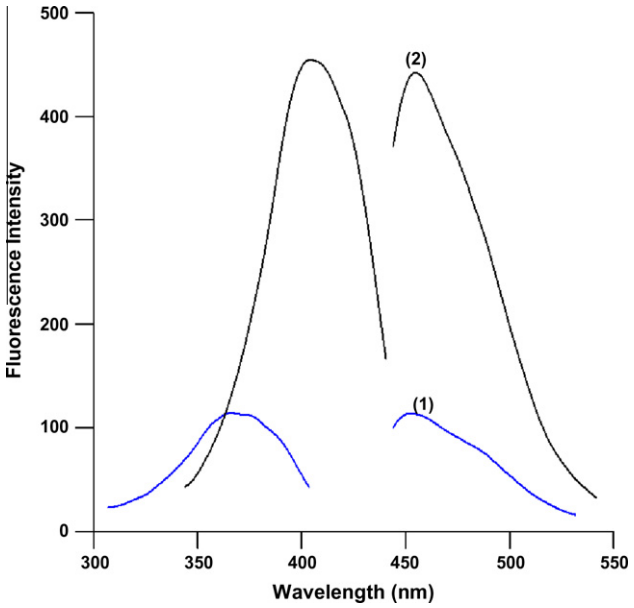
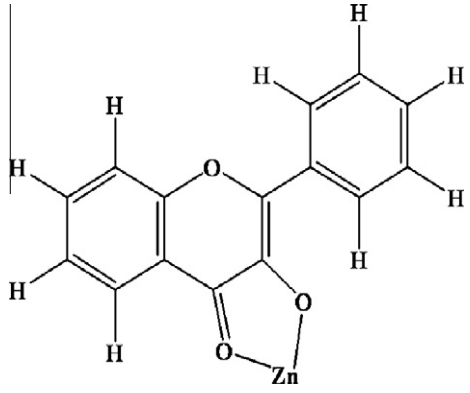


Figure 1 Excitation and emission spectra of 3HF and its Zn complex in methanol. (1) Absence of zinc ions (2) with 1.1×10^{-7} M of zinc ions, [3HF] = 1.0×10^{-7} M, λ_{ex} = 378 nm, λ_{em} = 453 nm for 3HF ligand and λ_{ex} = 408 nm, λ_{em} = 478 nm for Zn complex.

solution of 3HF the fluorescence intensity rose considerably compared to that of the ligand alone. This significant enhancement of the emission was accompanied by a noticeable red shift in excitation and emission wavelengths from 378 nm to 406 nm and from 453 to 478 nm, respectively.

The shift in wavelength implies complex formation between zinc and the 3-hydroxy-4 keto group of the 3-hydroxy flavone moiety (Scheme 1). This behaviour of the 3HF molecule was anticipated from previous reports that the α-hydroxy ketone functional group is capable of forming complexes with many metal ions (Dangleterre et al., 2008).

A relatively weaker signal of the fluorescence emission for 3HF, when compared to its complexed zinc counterpart, is due largely to the presence of hydroxyketone functional group in the molecule, which is known to undergo intramolecular proton-transfer processes. The enhancement in the fluorescence intensity that results upon complexation is attributed to the inhibition of the intramolecular proton transfer described above. Furthermore, coordination of the ligand to the metal ion increases the effective rigidity of the molecule and minimizes non-radiative dissipation processes.



Scheme 1 Proposed structure of Zn-3HF complex.

3.2. Complex stoichiometry

Since the fluorescence intensity results from the formation of the zinc complex, it was necessary to optimize the metal/ligand ratio to ascertain the maximum complexation of the metal. The optimum concentration of 3HF in methanol was accomplished by measuring the fluorescence intensity of various molar ratios of Zn^{2+} to 3HF ranging from 0.1 to 2. (The molarity of 3HF was maintained at 1.0×10^{-6} M while the molarity of the zinc ions was varied from 1.0×10^{-7} to 2.0×10^{-6} M). It can be seen from Fig. 2 that the fluorescence intensity increases with increasing the ratio of Zn^{2+}:3HF. On extrapolating the linear parts of the curves, the graphs intersect at a ratio of 1:1, implying that the stoichiometry of the complex involves one zinc ion and one ligand anion ($3HF^{-}$).

The stoichiometry of the complex was further studied by the continuous variation method (Fig. 3). The maximum fluorescence intensity was obtained at a mole fraction of Zn^{2+} of 0.5, corroborating that the ratio of Zn^{2+}:$3HF^{-}$ in the complex is 1:1.

Similar complexation behavior was previously reported by Cornard et al. (2006) who denoted that additional exogenous ligands are expected in the coordination sphere to satisfy the required geometry at the metal center. In addition, the presence of counter ions to neutralize the complex fulfils the desired stereochemistry.

3.3. Effect of pH

The pH of the reaction media has a great effect on the existing form of the reagent and hence affected the complexation of zinc by the ligand. Therefore, it was necessary to determine the pH of the system that produced the maximum complex formation and, in turn, the highest fluorescence intensity. The effect of the pH on the fluorescence properties of the Zn-3HF complex was thus studied by measuring the fluorescence of the complex in buffer from pH 3 to 11. Fig. 4 shows that on increasing the pH from 3.0 to 9.0 the fluorescence intensity increased and attains a maximum value at a pH of 7.5. A further increase in the pH resulted in a sharp decrease

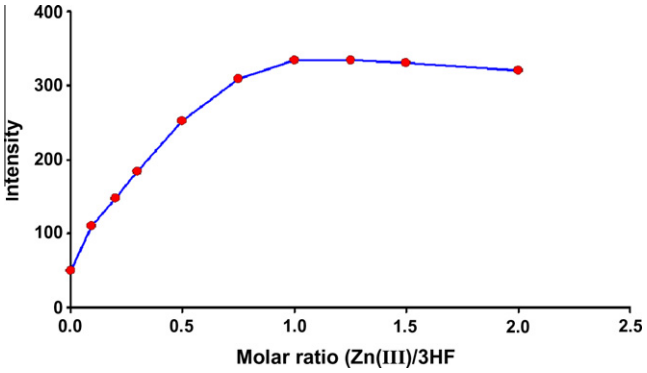


Figure 2 The fluorescence intensity of Zn (3HF) versus Zn (II) ions ratio in methanol. [3HF] = 1.0×10^{-6} M. λ_{em} = 478 nm, λ_{ex} = 408 nm.

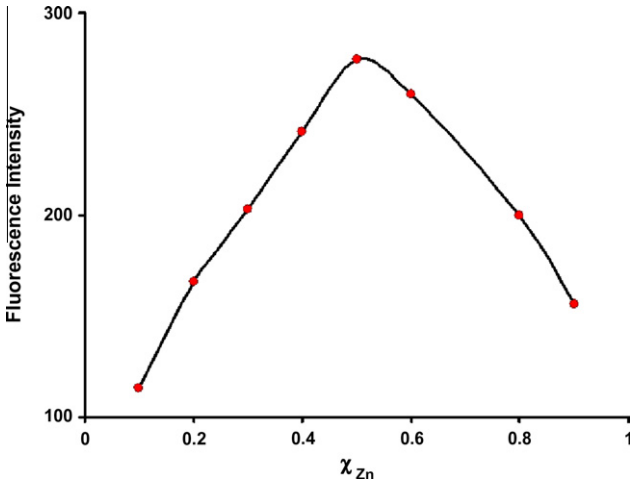


Figure 3 Determination of the stochiometry of Zn (3HF) complex by applying Job's method in methanol. λ_{ex} = 408 nm, λ_{em} = 478 nm. [Zn(II)] = [3HF] = 1.0×10^{-6} M.

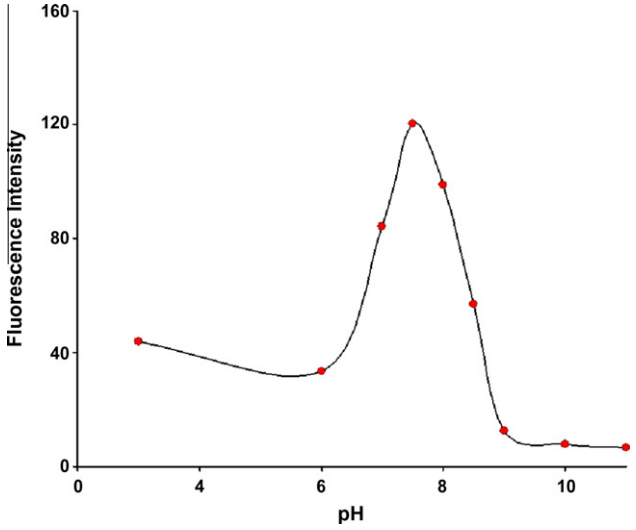


Figure 4 Influence of pH on fluorescence of Zn (3HF). [Zn(II)] ions 2.0×10^{-6} M, [3HF] = 1.0×10^{-5} M, [acetate buffer] = 0.2 M, λ_{ex} = 408 nm, λ_{em} = 457 nm.

in the fluorescence intensity. The effect of pH can be rationalized in terms of the stability of Zn-3HF. The acidic medium favors the dissociation of the complex and re-protonation of the hydroxyl group in the flavone ring, hence the reduction in the fluorescence intensity.

At higher pH, the concentration of OH^- increases, there is a competition between the OH^- ions and the ligand for the Zn^{2+} ion, which decreases the amount of the desired complex, thereby lowering the fluorescence intensity. Similar results were previously reported for the complexation behavior of Zn ions with 6-methoxy-8-p-toluenesulphonamido-quinoline (TSQ) ligand whereby an optimum pH of 7.4 was observed for complexation (Jiang and Guo, 2004). The optimum pH in our study was therefore maintained at 7.5 using Tris buffer. The fluorescence of 3HF in aqueous system revealed a band at 498 nm emission with an excitation at 413 nm. These results showed a red shift in both excitation and emission wavelength when compared to the spectra in methanol where an excitation of 378 nm and emission of 442 nm was observed (Fig. 1). Similarly, addition of zinc resulted in the fluorescence enhancement accompanied by a blue shift in both excitation and emission wavelength to 406 nm and 457 nm, respectively (Fig. 5).

3.4. Analytical characteristics/interferences/applications

Under the optimum experimental conditions described above a suitable linear relationship between fluorescence intensity and zinc concentration in the range 0–500 ppb was obtained. The minimum limit of detection was accordingly computed as 1.5 ppb. The reproducibility of the method was subsequently examined for 200 ppb standard solutions of zinc and the relative standard deviation was found to be less than 2% (n = 6) indicating that the precision was satisfactory.

A systematic study of the influence of interference of foreign ions on the determination of zinc was carried out. A solution containing 400 ppb of zinc was prepared in the presence of various amounts of Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, and Cu^{2+}. The results show little or no interference observed in the presence of 100 ppm of Ca^{2+} and Mg^{2+} (Table 1). However, Fe^{3+} and Cu^{2+} gave a decrease in recovery. The low recovery of zinc in the presence of iron and copper may be explained to be due to competition of these ions for 3HF ligand. This will result in a relatively less Zn-3HF complex being formed. Although both iron and copper are present in the multivitamin preparation, the detection limit of zinc ion by this method is low enough to ensure that the interference effect is eliminated by dilution during the analysis. Al^{3+} when present at 50 ppm gave a recovery value of 80.8 ± 0.9%. However, this is not expected to pose a serious interference in drinking water samples because the expected amount of Al^{3+} in drinking water is 200 ppb (Lucena et al., 1994). Furthermore, Al^{3+} is not ex-

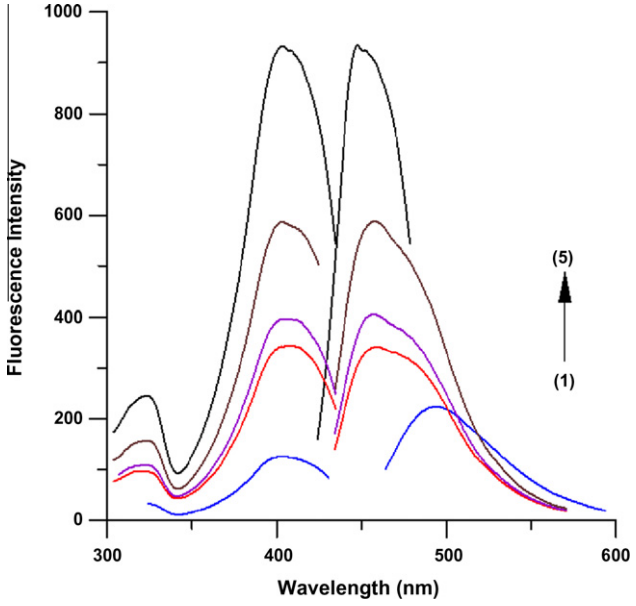


Figure 5 The effect of varying the concentration of zinc on fluorescence intensity of Zn (3HF). [3HF] = 1.0×10^{-5} M, pH = 7.5, [Zn(II)]: (1) 0, (2) 5.0×10^{-8} M, 1.0×10^{-7} M, (4) 5.0×10^{-7} M, (5) 1.0×10^{-6} M.

Table 1 Recovery studies of zinc.

Sample	Recovery ± RSD%
Deionized water Zn (400 ppb)	98.9 ± 1.3
Zn (400 ppb) + Ca (100 ppm)	98.6 ± 0.6
Zn (400 ppb) + Mg (100 ppm)	89.9 ± 0.8
Zn (400 ppb) + Al (50 ppm)	80.8 ± 0.9
Zn (400 ppb) + Fe (10 ppm)	32.2 ± 1.1
Zn (400 ppb) + Cu (10 ppm)	75.2 ± 1.4
Tap water	
(300 ppb)	99.1 ± 0.7
(100 ppb)	94.7 ± 1.2
Pharmaceuticals	
Multi vitamin tablets (7.5 mg/tablet)	97.3 ± 0.7
Multi vitamin capsule (4 mg/capsule)	98.8 ± 2.2

pected to be present in pharmaceuticals or hair shampoo under normal circumstances and thus should not be a serious problem in the determination of zinc.

The method was subsequently applied to the determination of Zn ions in tap water, multivitamin tablets/capsules. The results obtained are shown in Table 1. It can be seen that satisfactory recoveries of zinc were obtained with values ranging from 90% to 99% indicating that there was no evidence of interference from the matrix.

3.5. Independent validation of the method

Tablets/capsules, shampoo and water samples with five independent determinations were analyzed using the proposed fluorimetric method and the flame-AAS procedure as a reference method. The results are shown in Table 2. Both methods reported reasonable recoveries and relative standard deviations. The results obtained clearly suggest that the method developed is suitable for the determination of Zn ions in tap water, multivitamin tablets/capsules and shampoo samples without fear of interferences from the matrix. The results obtained by the two methods are compared statistically as shown in Table 2. It can be inferred from the statistical parameters of Table 2 (Student's *t*-test and *F*-test) that the two methods exhibit comparable accuracy and precision. The proposed method on the other hand can be applied in routine analysis as an alternative to flame-AAS method. The greater sensitivity of the proposed method over flame-AAS makes it advantageous when low zinc concentrations need to be determined. Although the addition of reagents makes the proposed method slightly more laborious than AAS, this step is only a small part of the overall procedure.

Table 2 Analysis of zinc by the proposed method and by the standard FAAS method.

Sample	Proposed method (ppm)	AAS method (ppm)	*t* calculated	*F* calculated
Tap water	0.36	0.38	0.89	4.0
Shampoo	97.4	100.4	1.03	3.7
Tablet	0.58	0.61	1.20	3.8
Capsule	0.89	0.94	1.52	3.5

t table = 2.31 ($n = 5$, $\alpha = 0.05$), *F* table = 6.36 ($n = 5$, $\alpha = 0.05$).

4. Conclusion

The development of a sensitive and selective fluorimetric method has been described for the determination of Zn ions based on the complexation with 3HF in Tris buffer pH 7.5. The proposed method was applied to the analysis of Zn ions in water samples, pharmaceutical tablets/capsules and hair shampoo samples and was found to exhibit better sensitivity than flame-AAS. The greater sensitivity of the proposed method over flame-AAS makes it advantageous for trace determination of zinc ions in various samples and without interference from other ions present in the matrix.

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